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# Effect of pH on Freshwater Cyanobacteria Isolated from Different Habitats of Southern Karnataka

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**ABSTRACT:**
In the present study, the influence of pH on the growth of six species of cyanobacteria has been carried out. Among physical factors pH is a very important factor which influences their occurrence and diversity. By this study it was also found that *Microcystis aeruginosa* (bloom forming cyanobacteria) which showed growth at highly alkaline pH (9.0 to 10.0). *Phormidium chlorinum* (isolated from sewage water) was able to grow at acidic pH (5.0 to 6.5). But overall study showed that neutral to slightly alkaline pH (7.0 to 8.5) is the optimum range for good growth of most of the cultured species. It was also revealed that species *Geitlerinema calcuttense* of dairy effluents showed good growth at acidic to alkaline pH. *Scytonema bohnerii* isolated from a sulfur spring prefer to grow at slightly acidic pH (6.5). The overall study showed cyanobacteria generally prefer neutral to alkaline pH for their optimum growth. It was found that, cyanobacteria under acidic to neutral pH (5.5 to 7.0) can synthesize higher carotenoids rather than under alkaline pH. The study indicated that, they can produce higher phycobillin pigments in terms of phycocyanin, allo-phycocyanin and phycoerythrin at slightly alkaline pH (8.0 to 8.5). The study also revealed that cyanobacteria produce higher phycoerythrin pigment at slight alkaline pH between 7.5 and 8.0 except the species, *Microcystis aeruginosa* where it showed higher phycoerythrin content at pH 9.0. The overall study revealed that as the pH value increased, there was an increase in the phycobilin pigments in the cyanobacteria.

**Key words:** Freshwater, Cyanobacteria, pH, Biomass, Pigments, Southern Karnataka.

## INTRODUCTION

Cyanobacteria are a diverse group of prokaryotes and possess oxygen evolving photosynthetic system [1]. There are several physical and chemical factors which influence their growth, photosynthetic ability and development under natural conditions. These include physical factors like light, temperature, pH, water conductivity etc. and inorganic nutrients, organic matter and biological factors [2]. Among physical factors pH is a very important factor which influences their distribution and diversity and variation in pH affects their growth [3-5]. It can change the clay buffering system thus influencing the availability of trace metals and essential nutrients and inflict direct physiological effects at extreme levels [6].

The influence of pH on the photosynthetic apparatus of cyanobacteria has received some attention because pH is an important factor in determining the distribution of cyanobacteria in aquatic ecosystems [7,8]. Cyanobacteria are alkalophiles capable of maintaining an internal constant pH of 7.1-7.5 in the range of external pH from 5 to 10 [9]. Changes in pH affect the solubility and bioavailability of nutrients, transport of substances across the cytoplasmic membranes and the activity of intra- and extracellular enzymes as well as photosynthetic electron transport and the osmotic potential of the cytoplasm [10,11]. Cyanobacteria are generally reported to prefer neutral to alkaline pH for their optimum growth [12]. There are very few reports on their occurence at low pH (13-16].

In the present study, the influence of pH levels on the growth of six species of cyanobacterial isolates from different freshwater habitats is reported.

## MATERIALS AND METHODS

### *Cyanobacterial cultures*

The six species of cyanobacteria namely, *Oxynema acuminatum* (Gomont) Chatchawan, Komárek was isolated from a artificial tank at Malavalli of Manday District; *Phormidium lucidum* Kützing ex Gomont from Bhadra reservoir of Chickmagalore district; *Phormidium chlorinum* (Kützing ex Gomont) Umezaki & Watanabe from a sewage drain at Pumpwell near Mangalore city; *Scytonema bohnerii* Schmidle isolated from Panekal sulfur spring; *Geitlerinema calcuttense* (Biswas) Anagnostidis from dairy effluent; *Microcystis aeruginosa* Kuetzing from Kukkarahalli tank at Mysore District and they were cultured and maintained in BG-11 broth culture medium. About 5 ml of homogenized culture suspension of each of the 15- day old culture was inoculated into each of the 250 ml conical flasks containing 100 ml BG - 11 culture medium. The pH of the medium was adjusted to different levels, ranging from 5.0 to 12.0. For each culture, three replicates were maintained. The cultures were grown at

illumination (2000 lux) at a temperature of 28 ± $2^0$ C with 14:10 hour light: dark regime. The nitrogen free BG-11 medium was used for nitrogen fixing cyanobacteria. The growth in terms of chlorophyll- a was assessed after 20 days of incubation.

***Determination of biomass***

The biomass of the cultures was evaluated by dry weight method in different pH regime for 25 days [17]. 10 ml culture was concentrated by centrifugation at 3,000 rpm for 10 min. Pellet was washed by deionized water and centrifuged followed by drying (at 60°C). Dry weight was expressed as g/L. All experiments were conducted three times. Data were reported as the average value of three sets.

***Estimation of chlorophyll-a***

Chlorophyll-a was estimated by Jeffrey and Humphrey method [18]. Chlorophyll-a content was extracted with 90% acetone (v/v). About 10 ml of homogenized cyanobacterial suspension was pelleted by centrifugation and 10 ml of 90% acetone was added. The centrifuge tube was vigorously shaken so as to dissolve completely in the solvent. Then all the tubes were placed in a refrigerator for 24 hours for complete extraction of the pigments. After the extraction period, the samples were centrifuged and the supernatant was collected. The supernatant was made up to 10 ml with 90% acetone and absorbance measured at 665 nm in a UV visible spectrophotometer (Systronics India, Ltd.) against 90% acetone as blank. The amount was calculated using the extinction coefficient given by Jeffrey and Humphrey [18]. Mean values of triplicates ± SD were recorded.

The amount of chlorophyll-a was determined using the equation:

chlorophyll- a (µg/ml) = 11.85 $A_{664}$ –1.54 $A_{647}$ – 0.08 $A_{630}$

***Estimation of total carotenoids***

Carotenoids were estimated according to the protocol prescribed by Parsons and Strickland [19]. Carotenoids present in the samples were extracted using 80% acetone. After complete extraction, samples were centrifuged for about 10 minutes at 5000 rpm, and the absorbance of the clear solution was measured at 480 and 510 nm wavelengths using UV visible spectrophotometer, taking 80% acetone solution as the blank. The absorbance of the sample was also obtained at 750 nm, which was subtracted from the values at 480 and 510 nm, thus minimizing the error.

The amount of carotenoids was determined using the equation:

Total carotenoids (µg/ml) = 7.6 (E480 - E750) - 1.49 (E510 - E750)

***Estimation of phycobilin pigments***

Estimation of phycobilin pigments like phycocyanin, allophycocyanin and phycoerythrin were done by spectrophotometric method [20, 21]. The known volumes of cyanobacterial suspensions were centrifuged and the pellets were suspended in 5 ml of 50 mM phosphate buffer (pH 7.0). The contents were repeatedly frozen and thawed and centrifuged in order to facilitate complete extraction. The supernatants were pooled and the absorbance was measured at 565, 620 and 650 nm against phosphate buffer blank [22]. Calculations were done using the following equations given by Tandeau De Marsac and Houmard [23].

$$\text{Phycocyanin (PC) µg/ml} = \frac{A620 - (0.7 \times A650)}{17.38}$$

$$\text{Allophycocyanin (APC) µg/ml} = \frac{A650 - (0.208 \times A620)}{15.09}$$

$$\text{Phycoerythrin (PE) µg/ml} = \frac{A565 - 2.41\,(PC) - 0.849\,(APC)}{19.62}$$

**Statistics**

The data of dry weight, chlorophyll-a, carotenoids and phycobilin pigment contents were expressed as mean of the triplicate values ± standard deviation (SD). The obtained data was subjected to analysis of variance (ANOVA) and then followed by Bonferroni post hoc analysis was done (SPSS statistical software, version 21.0) in order to verify significant difference in dry weight, chlorophyll-a, carotenoid and phycobilin pigment contents in cyanobacterial species cultured at different pH levels.

**RESULTS**

Effect of different pH levels on the growth pattern of six species is tabulated in Table 1.1. The study has revealed that freshwater cyanobacteria prefer neutral to slightly alkaline pH for their growth (7.0 - 8.5), except sewage and sulfur spring isolates which prefer acidic pH

(6.5) for their optimum growth. The species did not show any sustained growth below pH 6.0.

Effect of different pH on the growth (biomass) in terms of dry weight measurements of the six species is given in Figure 1.1 (a). Similarly, effect of different pH on the growth in terms of chlorophyll-a of the six species is given in Figure 1.1 (b). It was noticed that species such as *Oxynema acuminatum* and *Phormidium lucidum* showed higher biomass and chlorophyll-a content at neutral pH (7.0 to 7.5) ($p<0.01$). *Geitlerinema calcuttense* isolated from dairy effluents showed good growth at acidic to alkaline pH (6.0-9.5) ($p<0.01$). The species namely, *Phormidium chlorinum* isolated from a sewage drain and *Scytonema bohnerii* isolated from the Panekal sulfur spring prefer to grow at acidic pH (5.5-6.5) ($p<0.01$). These species exhibited optimum growth at pH 6.5, whereas, *Microcystis aeruginosa* (bloom forming species) isolated from a natural lake preferred to grow at higher alkaline pH (8.0-10.0) and showed higher chlorophyll-a content at pH 9.5 ($p<0.001$).

Similar trend was noticed in the carotenoid contents in these six species (Figure 1.1.c). The species, *Oxynema acuminatum* and *Phormidium lucidum* showed higher carotenoid content at pH between 7.0 and 7.5. Similarly, *Geitlerinema calcuttense* showed higher carotenoid content between acidic to alkaline pH (6.0- 9.5) ($p<0.05$). The species namely, *Phormidium chlorinum* (isolated from sewage drain) and *Scytonema bohnerii* (sulfur spring) exhibited maximum carotenoids at acidic pH (5.5-7.0). These species showed significant carotenoids at pH of 6.5 ($p<0.001$). *Microcystis aeruginosa* showed optimum carotenoids at high alkaline pH (9.0 to 10.0) ($p<0.01$). The overall study indicated that, cyanobacteria under acidic to neutral pH (5.5 to 7.0) can synthesize higher carotenoids ($p<0.05$) rather than under alkaline pH.

Effect of pH on the phycobilin pigments in the six species is shown in Figure 1.2 (a-c). The study indicated that, cyanobacteria can produce higher phycobillin pigments in terms of phycocyanin, allophycocyanin and phycoerythrin at slightly alkaline pH (8.0 to 8.5) ($p<0.05$). It was observed that, among the six species studied, more phycocyanin content was noticed at pH 8.5 (Figure 1.2 a.) ($p<0.01$), except in *Microcystis aeruginosa* where it exhibited high rate of phycocyanin pigment at pH of 9.5 ($p<0.01$). Similar observation was made in the case of allophycocyanin contents in these species (Figure 1.2 b.) where the species exhibited maximum allophycocyanin composition at higher pH (9.0) ($p<0.01$), while *Microcystis aeruginosa* showed higher allophycocyanin content at pH 8.5 ($p<0.05$). Phycoerythrin content in the six species of cyanobacteria at different pH levels is shown in Figure 1.2 (c.). By this study it revealed that cyanobacteria produce higher phycoerythrin pigment at slight alkaline pH between 7.5 and 8.0 ($p<0.05$) except the species, *Microcystis aeruginosa* where it showed higher phycoerythrin content at pH 9.0 ($p<0.01$). In this study, *Scytonema bohnerii* showed significant amount of phycoerythrin content of 5.525 µg/ml at pH of 8.0 ($p<0.001$). The overall study revealed that as the pH value increased, there was an increase in the phycobilin pigments in the cyanobacteria.

**DISCUSSION**

Besides chlorophyll-a, cyanobacteria contain phycobiliproteins as the main photosynthetic pigments. These proteins are essential components of the phycobilisomes, which constitute the photosystem II light-harvesting complexes of cyanobacteria [24]. The composition of the photosynthetic apparatus of cyanobacteria is influenced by environmental factors such as pH, temperature, irradiance and $CO_2$ concentration [25].

From the above results it was found that, the optimum pH for their growth in culture appears to be similar to that of their natural habitats. This stressed the importance of location specific species which have adapted to their natural habitats. Similar trend was also observed [16]. They reported that acid tolerant cyanobacterial cultures produced higher growth and biomass than usual non acid soil isolates at acidic pH range.

Only one species viz., *Geitlerinema calcuttense* showed maximum chlorophyll-a content at pH 6.5. (2.35 µg/ml). Usually untreated dairy effluent having slightly acidic pH as reported by other researchers [26, 27]. Among sulfur spring species *Scytonema bohnerii* showed good growth at pH 5, 6 and 8. It indicates that this species is highly tolerant to variation in pH. The other two species showed very less amount of chlorophyll-a at acidic pH.

All species showed good growth up to pH 8.5. The sudden arrest of growth was observed above pH 10. Few investigators reported that alkaline pH favours the growth of cyanobacteria which results in algal bloom formation [28, 29]. The absence of chlorophyll-a content of the cells

below pH 5 in all the species tested indicated that pH has inhibitory effect on their growth. This effect is also proved by Gopalaswamy et al. (2002) [16].

Many investigators studied the combined effect of pH on the growth and pigment composition and other metabolic activities of various strains of cyanobacteria especially with nitrogen fixing species due to their wide application in the field of agriculture. Boussiba [30]. studied ammonia uptake in the alkaliphilic *Spirulina platensis* and found that uptake of ammonia was pH dependent with an optimum at pH 9.3. Poza-Carrion et al. (2001) [31] analyzed the combined effect of pH, irradiance and inorganic carbon availability on growth and pigment composition of *Nostoc* sp. strain UAM 206 isolated from rice fields. They reported that under inorganic carbon limitation the growth rate was affected by pH but not by irradiance; chlorophyll-a content was not affected by pH. However with increased pH, there was an increase in the phycobilin content [31, 32] which agrees with our results. Poza-Carrion et al. (2001) [31] showed that increase in pH (7 to 9) significantly increased the total phycobiliproteins content in *Nostoc* sp. UAM 206, which also supports our study where, pH value between 8.0-9.0 showed higher phycobilin pigments.

The pH is one of the factors in reducing the toxicity of herbicides and other pesticides as reported by some investigators [33, 34]. Their results suggest that high pH (7.0 - 9.0) are most effective in reducing the herbicide and pesticide toxicity compared to acidic pH by progressive increase in the growth yield of the cyanobacteria in terms of chlorophyll- a. The influence of pH on the growth of thermophilic cyanobacterium *Mastigocladus laminosus* in continuous culture was studied by Muster et al. (1983) [35]. They reported that under optimal conditions the filaments contained 8 cells with high phycocyanin content but at high pH 2 to 4 cell filaments containing little phycocyanin content. It suggests that pH also has effect on the cell morphology of the cyanobacteria. In another study with regard to the thermophilic cyanobacterium conducted by Chung-Ching et al. (2006) [36] showed that photosynthetic performance and growth of *Synechococcus lividus* isolated from Copeland, Taiwan were sensitive to fluctuations in temperature but not in pH. Similar result was obtained for *Scytonema bohnerii* with regard to pH in the present study.

The relationship between intracellular pH, growth characteristics and calcium content in *Anabaena* sp. strain PCC 7120 exposed to low pH was studied by Giraldez – Ruiz et al. (1997) [37]. They noted that inhibition of growth and oxygen evolution at pH below 6.0 in the presence of standard calcium concentration (0 - 25 mM). The organism was unable to maintain a relatively constant internal pH at pH 6.0 and below which led to acidification of cytoplasm. In another study conducted by Nayak and Prasanna (2007) [38] the influence of soil pH was evaluated on the abundance and generic diversity of cyanobacteria in soil samples collected from diverse rice field soils of India. A total of 166 forms including 130 heterocystous and 36 non-heterocystous forms were isolated and highest percentage of abundance of heterocystous forms was observed at pH 8.1. From the observations made by this study and the study conducted by other researchers indicates that cyanobacteria prefer neutral to alkaline pH for good growth in different habitats [39]. Several cyanobacterial cultures were isolated from acidic soils of Kerala having pH of 3.8 [14]. Sing et al. (1997) [40] isolated many acid tolerant strains of cyanobacteria from acid soils of Nagaland. The effect of acid tolerant species on rice variety ASD 20 was studied. The study revealed that the inoculation of acid tolerant cyanobacteria comprising *Anabaen* -AT-TGK-5C10, *Nostoc*-AT-TGK-5A4, *Oscillatoria* AT-TGK-5C9, *Westiellopsis* AT-TGK-4AT7 and *Westiellopsis* –AT-TGK-5A9 was found to be better in increasing the growth and yield of ASD 20 besides excreting maximum ammonia in the flood water [41].

In the laboratory cyanobacteria are grown at a pH close to neutral (7.0- 7.5) , but they are able to acclimate to a range of different pH conditions [42-44]. If the growth medium is not buffered, pH tends to increase over growth time [44,45]. In natural habitats cyanobacteria prefer alkaline environments. At a higher pH, the concentration of bicarbonate is high and the availability of inorganic carbon is less which is likely to limit cyanobacterial growth. Sensitivity to low pH might thus be in part due to the effects of carbon limitation. It is known that the most prominent changes in the proteome were restricted to the periplasm and the cytoplasmic fraction remained relatively untouched by pH stress [44]. This implies that the cell surface moderates the effect of pH stress before intracellular components are severely affected.

The cyanobacterial abundance was noted when the pH was raised. This agrees with our findings particularly for sewage isolate *Phormidium chlorinum* and spring isolate *Scytonema bohnerii*

where these two species exhibited higher chlorophyll-a composition at lower pH 6.5. Similarly, reduced growth was observed in a group of pH tolerant cyanobacteria when the pH exceeded 9.5 [46]. This also agrees with our results.

The decrease in cyanobacterial abundance in the presence of high alkaline pH is most likely due to the drastic change in their environment. This can be explained by their decreased ability to photosynthesize in basic water. The availability of carbon dioxide for photosynthesis decreases as alkalinity increases [47]. If the ability of cyanobacteria to photosynthesize is impaired as the pH is increased, their abundance will decrease in water with high alkalinity which also agrees with our findings where, high alkaline pH inhibited the growth of cyanobacteria.

The tested species of cyanobacteria exhibited good growth at pH between 7.0 and 8.5. This data is in agreement with the previous reports [3, 48]. The fact that, all species were able to grow in acidic medium (pH 6.5), reflects that cyanobacteria can adapt to variable pH conditions as suggested by earlier studies [49-52]. The cyanobacteria possess different mechanisms for maintenance of pH homeostasis depending upon their natural habitat [38, 49].

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**DECLARATIONS**

The authors acknowledge that the manuscript submitted is their own original work. All authors participated in the work in a substantive way. All authors have seen and approved the manuscript as submitted. The manuscript has not been published and is not being submitted or considered for publication elsewhere.

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Table 1.1: Growth pattern of six species of cyanobacteria in different pH conditions.

| species | pH range | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5.0 | 5.5 | 6.0 | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 | 9.5 | 10.0 | 10.5 | 11.0 |
| *Oxynema acuminatum* | - | + | + | + | + + + | + + + | + + | + + | + | + | + | - | - |
| *Phormidium lucidum* | - | + | + | + | + + + | + + + | + + | + + | + | + | + | - | - |
| *Phormidium chlorinum* | + | + | + + | + + + | + + | + + | + + | + | + | + | - | - | - |
| *Scytonema bohnerii* | + | + | + + | + + + | + + | + + | + + | + | + | + | - | - | - |
| *Geitlerinema calcuttense* | - | + | + | + + | + + | + + + | + + + | + + | + | + | - | - | - |
| *Microcystis aeruginosa* | - | - | + | + | + | + + | + + | + + | + + + | + + + | + + | + | - |

+ : slight growth; + + : moderate growth; + + + : good growth; - : no growth

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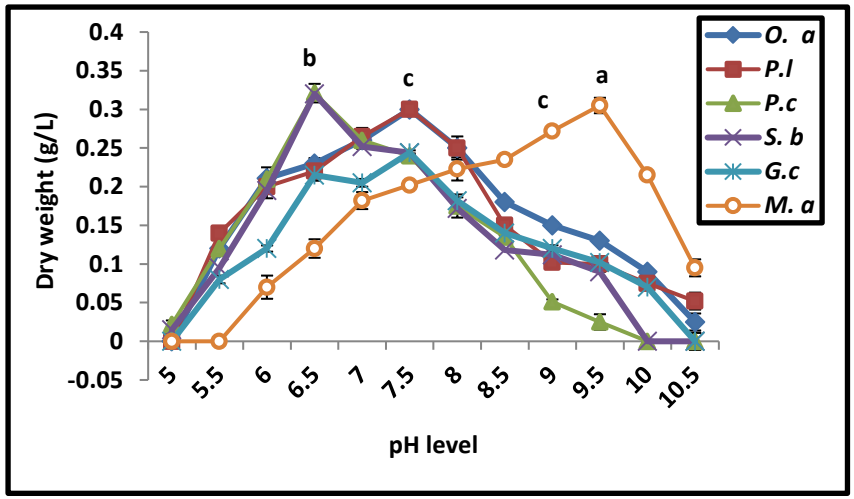
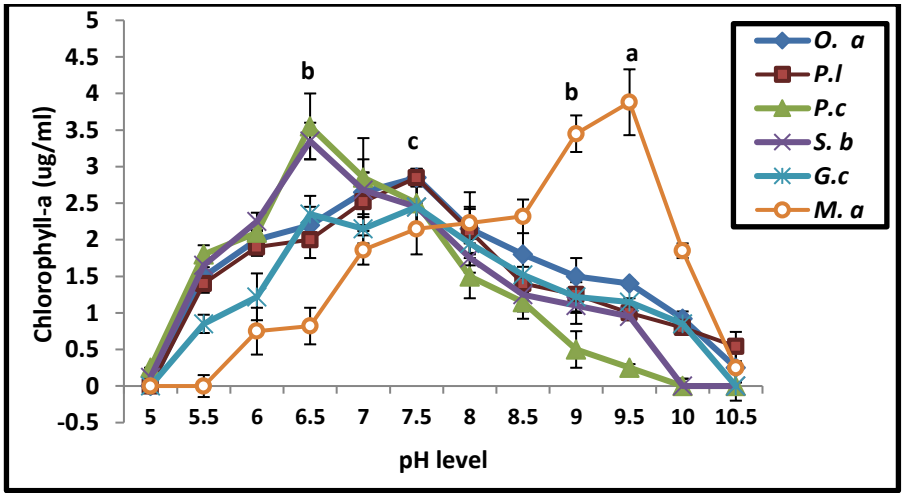
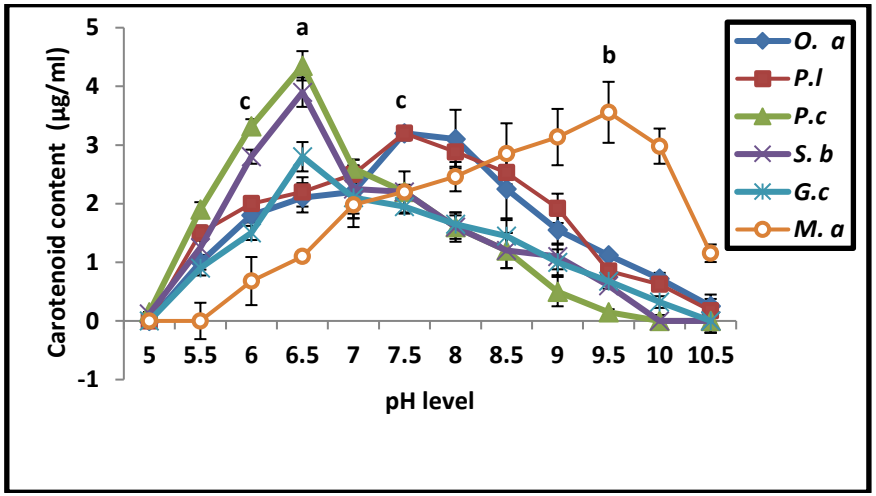



(a.)

(b.)




(c.)

Figure 1.1 (a-c ): Effect of different pH on the growth in terms of dry weight, chlorophyll-a and carotenoid content in six species of cyanobacteria* isolated from different habitats. *Mean of the triplicate values ± standard deviation.
[a] $p < 0.001$, [b] $p<0.01$, [c] $p<0.05$

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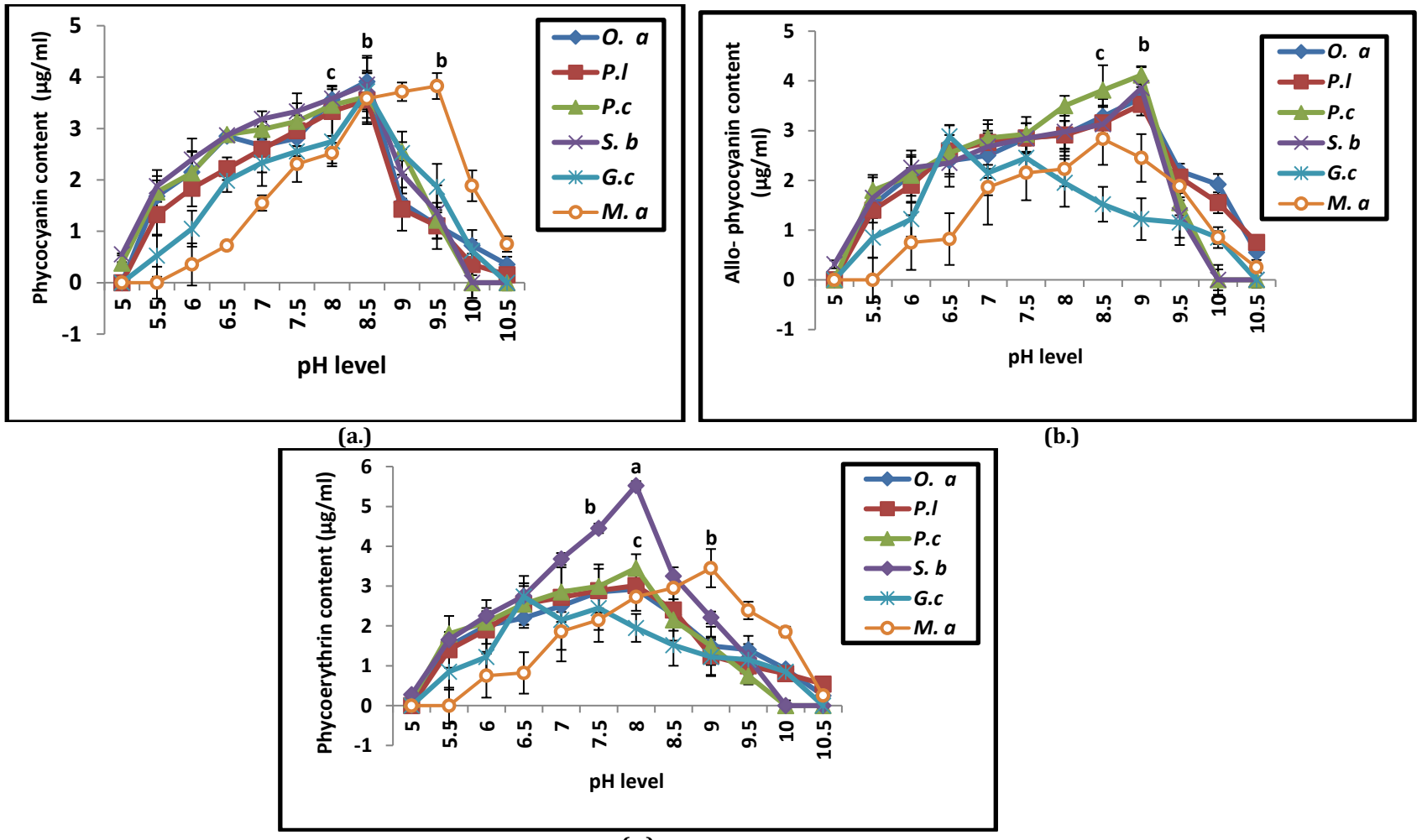


Figure 1.2. (a-c): Effect of pH on the phycobilin pigments in (phycocyanin, allo phycocyanin and phycoerythrin) six species of cyanobacteria*. *Mean of the triplicate values ± standard deviation. [a] $p < 0.001$, [b] $p<0.01$, [c] $p<0.05$
(* ***O. a***: *Oxynema acuminatum;* ***P. l*** : *Phormidium lucidum ;* ***P. c*** : *Phormidium chlorinum;* ***S. b***: *Scytonema bohnerii;* ***G. c***: *Geitlerinema calcuttense;* ***M. a***: *Microcystis aeruginosa*)